THE EFFECT OF METHYLGLYOXAL ON THE GLYCOLYTIC ENZYMES

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1. Introduction

Phenylglyoxal, glyoxal and methylglyoxal have been used to modify specifically, arginine residues in proteins [1–3]. Cheung and Fonda [4] have studied the effect of buffer and pH on the reaction of phenylglyoxal and methylglyoxal with free arginine and have found that the reaction rate, much faster with methylglyoxal than with phenylglyoxal, depends on the buffer type and increases by increasing the pH. Data on the inactivation mechanism of aldolase and glyceraldehyde-3-phosphate dehydrogenase by methylglyoxal have shown that one arginine residue is involved in the interaction of the enzyme with the ketoaldehyde [5,6]. Since all glycolytic enzymes, except triosephosphate isomerase, contain essential arginine residues [7–11], it seemed worthwhile to compare the effect of methylglyoxal on the enzymes of the glycolytic pathway.

2. Materials and methods

Substrates, enzymes and coenzymes were purchased from either Sigma or Boehringer Mannheim. Methylglyoxal (Fluka) freshly distilled and phenylglyoxal (Sigma), prepared every time before use were tested according to [12] and [13], respectively. Lactate dehydrogenase (EC 1.1.1.27) from rabbit muscle was assayed in the presence of 50 mM phosphate buffer (pH 7.0), 3.0 mM pyruvate and 0.2 mM NADH; alcohol dehydrogenase (EC 1.1.1.1) and hexokinase (EC 2.7.1.1) from yeast were tested according to [14] and [15], respectively. Phosphoglyceromutase (EC 2.7.5.3), enolase (EC 4.2.1.11), pyruvate kinase (EC 2.7.1.40), all from rabbit muscle and phosphoglycerate kinase (EC 2.7.2.3) from yeast were assayed following the procedure in [16]. Glucosephosphate isomerase (EC 5.3.1.9) from yeast was tested according to the procedure in [17]. Fructose 1,6 P_2 aldolase (EC 4.1.2.13) from rabbit muscle, triosephosphate isomerase (EC 5.3.1.1) from yeast and phosphofructokinase (EC 2.7.1.11) from rabbit muscle were assayed according to [18], [19] and [20], respectively. Glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.12) from rabbit muscle was tested in 50 mM pyrophosphate buffer (pH 8.5), 2.5 mM EDTA, 7.5 mM sodium arsenate, 0.5 mM NAD and 0.5 mM D-glyceraldehyde-3-phosphate. The inactivation experiments were carried out at 30°C with 2.5 mM methylglyoxal or phenylglyoxal in the presence of 50 mM veronal (pH 7.6) if not otherwise indicated.

3. Results and discussion

Kinetic studies carried out on alcohol dehydrogenase, lactate dehydrogenase, hexokinase, glucosephosphate isomerase, triosephosphate isomerase, pyruvate kinase and enolase have shown that methylglyoxal does not modify these enzymes: no loss of activity was observed when the enzyme was incubated with 2.5 mM methylglyoxal for 1 h at 30°C. The other glycolytic enzymes have been variously affected by methylglyoxal treatment, with half inactivation time ranging from 49.4 min for phosphofructokinase to 2.7 min for fructose 1,6 P_2 aldolase. Data reported in table 1 have shown the high specifity of methylglyoxal for aldolase, which is rapidly inactivated also at very low concentrations of inhibitors [5]. Comparing the effect of methylglyoxal and phenylglyoxal it has been demonstrated that aldolase and glyceraldehyde-3-phosphate dehydrogenase are more rapidly inactivated by the former than by the latter. An opposite behaviour was observed for phosphoglyceromutase, phosphoglycerate kinase and phosphofructokinase (table 2).

Table 1
Effect of methylglyoxal on glycolytic enzymes

Enzyme	τ (min)
Fructose 1,6 P_2 aldolase	2.7
Glyceraldehyde-3-phosphate dehydrogenase	19.8
Phosphoglyceromutase	20.0
Phosphoglycerate kinase	48.0
Phosphofructokinase	49.4

Enzymes were incubated at 30°C with 2.5 mM methylglyoxal in 50 mM veronal buffer (pH 7.6); except phosphofructokinase incubated in 50 mM phosphate buffer (pH 7.6). Enzymatic activities were always compared to controls subjected to the same treatment in absence of methylglyoxal. Data are expressed as half-inactivation time (τ), calculated from the inactivation constants

Table 2
Effect of methylglyoxal and phenylglyoxal on the inactivation rate of glycolytic enzymes

	τ (min)	
	Methyl-glyoxal	Phenyl-glyoxal
Fructose 1,6 P_2 aldolase	2.7	11.9
Glyceraldehyde-3-phosphate dehydrogenase	19.8	91.0
Phosphoglyceromutase	20.0	4.7
Phosphoglycerate kinase	48.0	11.0
Phosphofructokinase	49.4	4.8

Experiments were carried out in 2.5 mM methylglyoxal or phenylglyoxal as in table 1

The chemical structures of these ketoaldehydes could explain the different behaviour observed. Moreover phenylglyoxal is a chemical reagent and methylglyoxal can be considered a compound present in biological material [21], strictly related to the triose phosphate breakdown [22–27].

Acknowledgements

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